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Decoding Dopamine Signaling

Dopamine is a key neurotransmitter that is important for many physiological functions including motor control, mood, and the reward pathway. In this issue of *Cell*, the laboratories of Marc Caron and Li-Huei Tsai identify two very different molecules—β-arrestin 2 and Par-4, respectively—that unexpectedly are involved in dopamine signaling via the D2 receptor. These two new signaling pathways mediate the actions of dopamine on behavior and facilitate crosstalk between different signaling pathways that are activated by binding of dopamine to the D2 receptor.

The neurotransmitter dopamine is important for many physiological functions including motor control, mood, and the reward pathway. Many of these functions are integrated by the medium spiny neurons of the striatum, which lie below the cortex in the brain and respond to dopamine. Dopamine exerts its effects on neurons through five known subtypes of dopamine receptor (D1, 2, 3, 4, and 5). When dopamine binds to Gαs-coupled D1 and D5 receptors, the enzyme adenylate cyclase is activated and the secondary messenger cAMP is produced. In contrast, when dopamine binds to the Gαi/o-coupled D2, D3, and D4 receptors, adenylate cyclase activity is blocked and cAMP production is reduced (Neve and Neve, 1997). Neurons in the midbrain project their axons to the striatum and release dopamine, which modulates cAMP production by activating D1 and D2 receptors expressed by striatal neurons. These receptors work antagonistically to modulate synthesis of cAMP. Striatal neurons also receive input from neurons in the cortex that release the excitatory neurotransmitter glutamate. This results in stimulation of AMPA and NMDA ligand-gated ion channels and increases the intracellular concentration of Ca^{2+}, leading to activation of signaling pathways dependent upon this second messenger. Among the downstream effectors of cAMP and Ca^{2+} are DARPP-32 and RCS (regulator of calmodulin signaling), which integrate signals from both of these second messengers (Rakhilin et al., 2004). Characterization of these signaling pathways and their downstream effectors has provided insight into the regulation of the excitability and responsiveness of striatal neurons. Less is known about signaling events that take place upstream of these secondary effector molecules.

Communication in the dopamine system is particularly important because a variety of neurological and neuropsychiatric disorders, including schizophrenia, attention deficit hyperactivity disorder, Tourette syndrome, obsessive-compulsive disorder, Parkinson's disease, Huntington's disease, and drug addiction, result from impaired dopamine receptor signaling. Many of the drugs used to treat these disorders target dopamine receptors. For example, D2 antagonists such as haloperidol and risperidone are effective at reducing psychosis. Work with these and other synthetic dopamine receptor ligands indicates that there is more to blocking D2 receptor activity than reducing the intracellular cAMP concentration, and that the signaling pathways that make these drugs effective remain to be fully elucidated.

There are several barriers to elucidating the signaling pathways elicited by binding of dopamine to its D2 receptor. The receptors are polymorphic and are not particularly abundant, and mono-specific antibodies against D2 receptors have been difficult to obtain. D2 receptors are G protein-coupled receptors (GPCRs) containing the characteristic seven transmembrane regions and three N-linked glycosylation sites (see Figure 1). They form dimers with each other and occur as either long (mostly postsynaptic) or short (mostly presynaptic) isoforms that differ by 29 amino acids in the third cytoplasmic loop.

A panoply of downstream effectors in addition to cAMP and Ca^{2+} are activated when dopamine binds to D2 receptors. These include agents, such as Ca^{2+} channels, AMPA receptors, NMDA receptors, and phospholipase C (PLC), that influence the Ca^{2+} concentration. Yet other D2 effectors are downstream of Ca^{2+}, such as calcineurin (PP2B) and the β and ϵ isoforms of protein kinase C. Still other effector molecules may be activated by D2 receptors independently of Ca^{2+}: for example, Na^+ channels, inwardly rectifying K^+ (GIRK) channels, the Na^+/H^+ exchanger, protein phosphatase-1 (PP1), glycogen synthase kinase 3 (GSK3), the Raf/MEK/ERK1/2 pathway, phosphoinositol 3-kinase (PI 3-kinase), and transcription factors such as CREB, nuclear factor-κB (NF-κB), and serum-responsive element (Missale et al., 1998; Takeuchi and Fukunaga, 2004). Furthermore, D2 receptors interact with the Ca^{2+}-permeable GluR2 subunit of AMPA receptors and in this way influence Ca^{2+} signaling (Zou et al., 2005).

Some of these downstream effects depend on the Gαi/o subunit of the D2 receptor, whereas others are controlled through the Gβγ subunit. Other downstream effectors are modulated by G protein-independent signaling mechanisms, and some are controlled by both G protein-dependent and -independent mechanisms. Interactions of G proteins with the D2 receptor are coordinated by short peptide sequences near the amino and carboxyl termini of the receptor's third cytoplasmic loop, leaving the remaining portion free to interact with other proteins (see Figure 1). For example, the third cytoplasmic loop binds to the postsynaptic scaffolding

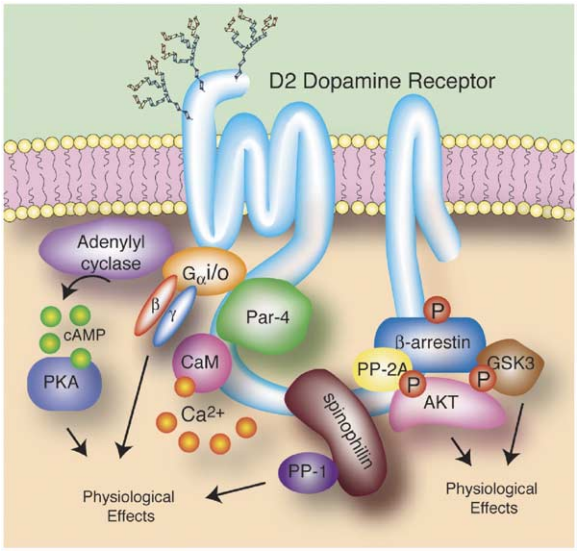

Figure 1. The D2 Dopamine Receptor and Its Downstream Signaling Machinery

Binding of dopamine to the D2 receptor results in activation of the Gαi/o protein coupled to its third cytoplasmic loop. This inhibits the ability of adenylate cyclase to synthesize cAMP and thereby activates PKA (left). Park et al. (2005) report that D2-dependent regulation of cAMP depends on the association of the D2 receptor with Par-4, an interaction that is competitively inhibited by activated Ca^{2+}-associated calmodulin. Beaulieu et al. (2005) describe a complex containing β-arrestin 2, PP-2A, and Akt (right) that mediates the effects of D2-receptor activation independently of Gαi/o-coupled mechanisms. Components of this signaling complex are regulated by phosphorylation/dephosphorylation events. The Akt substrate GSK3 may also be a part of this complex. Interactions between the D2 receptor, spinophilin, and PP1, as well as between Gαi/o and Gβ/γ, are also depicted.

protein, spinophilin, linking it to PP1, the actin cytoskeleton, and possibly other transmembrane proteins. Like other GPCRs, D2 receptors undergo desensitization, clathrin-mediated sequestration, and resensitization, presumably via the actions of G protein-coupled receptor kinases, arrestins, and components of the endocytic pathway, such as dynamin-2, which bind to the receptor (Macey et al., 2004). β-arrestin also acts as a scaffold for signaling complexes, coupling the D2 receptor to the Raf/MEK/ERK2 signaling pathway.

As more pathways radiating from activated D2 receptors are revealed, it is clear that these receptors encode an incredibly complex program of signaling cascades. In this issue of *Cell*, Park et al. (2005) and Beaulieu et al. (2005) reveal interactions of D2 receptors with two new and unexpected signaling pathway components. In their study, Park and colleagues demonstrate that the proapoptotic protein Par-4 (prostate apoptosis response 4) interacts with the third cytoplasmic loop of the D2 receptor. They demonstrate that this interaction is physiological and that Par-4 and D2 receptors colocalize in the synaptic membranes of striatal neurons. Furthermore, this interaction, which involves the leucine zipper domain of Par-4, is essential for Gαi/o-mediated inhibition of cAMP activity. The region of the D2 receptor that interacts with Par-4 contains a calmodulin binding domain, and Ca^{2+}-activated calmodulin competes with Par-4 for this site. This discovery is important, as the authors demonstrate that Gαi/o-dependent D2 regulation of gene expression dictated by the transcription factor CREB depends on an equilibrium between binding of Par-4 and of calmodulin to the D2 receptor. Increases in the intracellular Ca^{2+} concentration, possibly in response to activation of the D2 receptor, could result in displacement of Par-4 and uncoupling of the D2 receptor from Gαi/o, thereby providing negative feedback on D2-mediated cAMP attenuation. In turn, the resulting elevation of cAMP levels could provide a second round of feedback by attenuating some of the effects of Ca^{2+} signaling. This group also demonstrates that mice deficient in Par-4 exhibit specific behavioral phenotypes reminiscent of depression; these depression-like behaviors are responsive to antidepressant drugs of the selective serotonin reuptake inhibitor (SSRI) class. Thus, this signaling pathway may serve as a critical point for integrating serotonin and dopamine inputs on striatal neurons expressing D2 receptors.

Meanwhile, Beaulieu and colleagues had discovered that prolonged activation of D2 receptors in mice lacking the dopamine transporter (DAT)—which prevents dopamine removal from the synaptic cleft—inactivates protein kinase B (Akt) in a cAMP-independent fashion (Beaulieu et al., 2004). Akt is activated by phosphorylation of its serine residues 308 and 473. Akt binds to phosphatidylinositol-3,4,5-P_3 (PIP_3) at the plasma membrane and its activation is associated with PI3-kinase activity. Once bound to the membrane, Akt can be activated by phosphoinositide-dependent kinase 1 (PDK1), which also binds to PI3-K. Activated Akt phosphorylates a number of target proteins including GSK3, resulting in its inhibition.

Beaulieu et al. (2005) realized that there must be a connection between D2-receptor signaling and Akt because raclopride, a D2 receptor inhibitor, disrupted Akt inactivation. Furthermore, they observed that inhibition of GSK3 or activation of Akt in response to lithium or other GSK3 inhibitors reduced dopamine-associated increases in locomotor behavior in DAT-deficient mice or in wild-type mice treated with amphetamines, which elevate synaptic dopamine for prolonged periods. Moreover lithium, a GSK3 inhibitor that also activates Akt, could antagonize dopamine-dependent locomotor hyperactivity in these same mice. The investigators have now identified β-arrestin 2 as the biochemical connection between D2 receptors and Akt (Beaulieu et al., 2005). Using animal models coupled with behavioral and biochemical assays, they show that activation of D2 receptors induces the formation of a signaling complex involving β-arrestin 2, Akt, and protein phosphatase-2A (PP2A) that mediates the effects of dopamine. Dopamine-related behaviors were consistently attenuated in mice lacking β-arrestin 2, despite the fact that cAMP-dependent signaling remained intact. Furthermore, in animals lacking β-arrestin 2, amphetamine was unable to dephosphorylate and inactivate Akt. These results precisely identify β-arrestin as the bridge between D2 receptors and downstream effectors of Akt and show the importance of this new pathway in medi-

ating dopamine-dependent functions. Interestingly, in cultured neurons, D1 receptors can also activate Akt via a cAMP-dependent pathway (Brami-Cherrier et al., 2002). It will be important for future work to delineate which aspects of this pathway are under the control of D1 or D2 receptors, or both.

The two new studies expose hidden insights into dopamine signaling via D2 receptors, with their revelations of new functions for Par-4 and Akt. These proteins are associated with quite different cellular processes: Par-4 is a proapoptotic factor implicated in neurodegenerative disorders such as Alzheimer's disease (Xie and Guo, 2005), whereas Akt activity prevents cell death (Brunet et al., 2001). An emerging theme seems to be that components of the programmed cell death pathway are commandeered as mediators of neurotransmission and synaptic plasticity in the brain.

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